Remarks

Claims 30-33 and 35-39 and 41-76 remain in this application. Claim 40 has been cancelled. Claims 30, 33, 35, 36, 37, 39, 41, 44, 48, 50 and 51 have been amended, and new claims 66-76 have been added. The remarks below are intended to address each and every issue raised in the Office Action of September 6, 2000. Although that Action specifically addresses only claims 30-33 and 35-65, the arguments below apply equally to those claims in addition to the new claims presented herein.

Claim rejections under 35 U.S.C. §112, first paragraph

Claims 30-33 and 35-65 are rejected under 35 U.S.C. §112, first paragraph as not being enabled by the specification. Specifically, the Office states that the claims are not enabled for the use of any microbial cell administered by any means of any amount for any antigen to induce a protective immune response against the microbial cell or antigen it expresses.

With respect to the scope of microorganisms that are enabled as host cells by the instant specification, applicants point out that the claims have been amended to replace the term "microbial cell" with "bacterial cell," and to replace "immunoprotection" with "immune response." Applicants contend that the specification provides sufficient guidance that the skilled artisan would have been able to practice the claimed methods using any bacterial cell at the time of filing. The methods require only that vectors be introduced into the host cell that carry the essential and lethal genes and a gene that encodes an antigen. Such vectors could, at the time of application, be produced using standard and well known methods. The specification provides working examples using both *S. typhimurium* and *E. coli*. The skilled artisan would expect such methods to be applicable to any strain of bacteria.

The instant specification describes each of the elements necessary to practice the claimed methods in sufficient detail to enable the skilled artisan to practice the methods using any bacteria strain. Each of these elements will be discussed below with reference to the sections of the specification where the description is contained.

The system first requires a regulated essential gene. Essential genes are described at pages 11 through 16 of the specification. As defined therein, an essential gene is a gene required for cell viability. Such genes include genes necessary for metabolism or growth of the host cell; for example, genes encoding DNA ligase and gyrase, which are required for nucleic acid replication. All bacteria comprise genes in this category, as DNA replication is essential to the viability of all bacteria. Genes essential for cell wall or cell membrane integrity make up another particularly useful category of essential genes. As discussed at pages 13-16 of the instant specification, all bacteria have both a peptidoglycan layer of the cell wall and a cell membrane which serves to retain the contents of the cytoplasm. Genes that encode components of the cell wall and the enzymes that catalyze the biosynthesis of the cell wall

components are known in the art, as are genes involved in metabolism of cell membrane components. Aside from the essential genes described in the specification, the skilled artisan would have no difficulty in identifying genes that would be useful as the essential gene component of the claimed methods, and could do so simply by referring to the extensive body of literature in the prior art that addresses such genes.

In some embodiments, the system also comprises a regulated lethal gene. Lethal genes are discussed at pages 16 through 17 of the instant specification. As used in the context of the claimed methods, a lethal gene is one whose expression is lethal to the host cell. The prior art is full of examples of such genes, which include genes that encode, for example, products that function to form holes in cell membranes, nucleases, phospholipases and endolysins, all of which are discussed in the instant specification, among others. Aside from the lethal genes discussed in the specification, the skilled artisan would be able to identify numerous suitable lethal genes simply by referring to the extensive prior art literature that addresses such genes.

Next, the genes discussed above must be under the control of regulatory elements that enable the essential and lethal genes to be differentially expressed in permissive versus non-permissive environments such that the cell is viable in the permissive environment and not viable in the non-permissive environment. A number of different strategies for accomplishing such control are discussed in the specification at pages 19-22. Applicants point out that the methods discussed in that section are well characterized in the literature, and appropriate references that are indicative of the state of the art are cited in the text. Thus, various methods of regulating expression of the essential and lethal genes are available to the skilled artisan, in addition to the specific embodiments discussed in the Examples.

Vectors are also well known in the art, as discussed in the specification at pages 23-29, where tables (Tables 1, 2 and 3) are presented that describe various vectors that are useful in, or useful for constructing, an ELVS. The construction of vectors useful in the claimed methods is carried out using conventional techniques of molecular biology and recombinant DNA technology. Numerous references are cited in the text of the specification which exemplify the state of the art at the time of filing.

Expression of an antigen is also contemplated within the scope of the claimed methods, as discussed at pages 36-39 of the specification. Selection and recombinant expression of antigens is described in the prior art, such that the skilled artisan would expect that any known antigen can be expressed in the host bacteria. At the time of filing, the literature in the art was full of examples of antigens, and examples of host organisms that express heterologous antigens.

An appropriate host bacteria strain is also required to practice the methods of the claimed invention. Again, applicants point out that the literature of the art is not lacking in examples of bacterial

cells that colonize in an animal, and that can be manipulated using the methods described in the instant specification.

The claims at issue here are directed to a method of inducing an immune response in an animal by administering a bacterial cell that expresses an antigen, and also comprises an environmentally limited viability system. As discussed above, many of the components of the system are well known in the art. The essence of the invention is that the organism (bacterium) that is administered to the animal is capable of surviving within the animal, but is not viable outside of the animal. One of the drawbacks of the live bacterial vaccines taught in the prior art is that the organism utilized for delivery of an antigen is capable of surviving outside of the intended host animal. Thus, bacteria that express an antigen for the purpose of inducing an immune response can be introduced into the environment by, for example, excretion of feces containing the bacteria. Those bacteria then can potentially enter and colonize an unintended host animal. In some cases, such a transfer from one host animal to another is undesirable. The present invention addresses this issue. In the methods of the instant claims, the bacteria comprise an essential gene under the control of an expression mechanism whereby the gene is expressed when the bacteria is inside of the animal and not expressed when the bacteria is outside of the animal. In some embodiments, the methods also contemplate an environmentally limited viability system comprising an essential gene as above, and a lethal gene under the control of an expression mechanism whereby the gene is not expressed when the bacteria is inside of the animal and is expressed when the bacteria is outside of the animal. The claims are not directed to any particular essential gene, lethal gene, control mechanism, bacterial host, vector, antigen, or combination of these elements. Each of these elements, individually, is known to the skilled artisan. The invention claimed utilizes a combination of these elements in a way that was not known or suggested by the prior art. Again, the concept that the claims encompass is that of eliciting an immune response in an animal by administering a bacteria that expresses an antigen, wherein the bacteria also comprises an ELVS, so that the potential for accidentally immunizing an unintended animal, or releasing the bacteria into unintended environments is eliminated. This biological containment mechanism was unknown prior to the disclosure by the present inventors. The skilled artisan would have no reason to believe that such a containment system would not have applicability utilizing a wide range of essential genes, lethal genes, antigens, bacteria, control mechanisms and vectors. The specification provides working examples utilizing *Salmonella*. However, within the context of the state of the art at the time of filing, the skilled artisan would, without undue experimentation, have been able to practice the claimed methods utilizing various combinations of elements including various bacterial host cells, all of which could be found in the prior art or by utilizing methods known in the art, with a reasonable expectation of success. Therefore, the claims meet the enablement requirements of 35 U.S.C. §112, first paragraph.

In light of the above discussion, applicants respectfully request reconsideration and withdrawal of these rejections.

Claim 61 is rejected under 35 U.S.C. §112, first paragraph, as containing a required element that does not appear to be readily available. The Office Action alleges that a repeatable method for obtaining the pMEG-104 plasmid is not set forth in the specification.

Applicants point out that the plasmid vector pMEG-104 is shown in a diagram in Figure 4. In addition, Example 1 describes the method by which that plasmid vector was constructed, using conventional recombinant DNA methods known to the skilled artisan. All of the various components of the plasmid are described in the prior art, and are thus available to the public. Therefore, as the individual components of the plasmid are publicly available, and the specification sets forth the method used to assemble the components, the plasmid vector pMEG-104 is available to the public, and the claim therefore meets the enablement requirement of 35 U.S.C. §112.

Rejections under 35 U.S.C. §112, second paragraph

Claims 30-33 and 35-64 are rejected under 35 U.S.C. §112, second paragraph as being indefinite. Specifically, it is alleged that those claims recite an incomplete method because only one step, administering the composition to an animal, is recited. Applicants contend that the claims recite a complete method.

The specification, at pages 36-41, discusses the use of an organism (bacteria) comprising an environmentally limited viability system for delivery of an antigen for the purpose of eliciting an immune response. Therefore, it is clear based upon a reading of the disclosure that the immune response is based upon the expression of an antigen. Administration of the bacteria described in the claims, which bacteria expresses an antigen, will elicit the desired immune response. Therefore, the methods claimed require only one step, and as such are complete.

Claims 43, 44, 47, 51, 53-55 and 60 are objected to as reciting abbreviations without the abbreviation first being defined in the claims. Applicants point out that the alleged abbreviations are in actuality the names of particular genes. These genes are described in the specification such that a skilled artisan would understand exactly what these genes are and what they encode. Also, these are genes that are well known in the art, and are referred to therein by reference to the names. Therefore, applicants respectfully request withdrawal of these rejections.

Claim 51 is rejected as being indefinite for reciting the phrase "and promoter elsewhere in the cell," and an improper Markush group. Please note that the claim has been amended in order to eliminate both of the cited phrases and to make the claim clearer. In light of this amendment, applicants request that this rejection be withdrawn.

Double Patenting

It is noted in the Office Action that should claims 30 or 31 be found allowable, claims 12 or 13 will be objected to as being substantially duplicative. Applicants note that claims 12 and 13 are no longer pending in this application, and therefore that objection is moot.

Rejections under 35 U.S.C. §102

Claims 30-33, 35-38, 48-49 and 65 are rejected under 35 U.S.C. §102(b) as being anticipated by Nakayama et al. (1998) or Curtiss et al. (1989). Nakayama et al. is cited as disclosing a microbial cell wherein the loss of the *Asd*+ plasmid would result in cell death outside the host animal in the absence of supplemental DAP. Curtiss et al. (1989) is cited as disclosing essentially the same microbial cell.

The above-cited claims are not anticipated by the cited references because both references fail to teach all of the limitations of the claims. Please note that one of the critical features of the claimed invention is that the essential gene is under the control of a mechanism that allows the essential gene to be expressed inside of the animal, but not outside of the animal. The cited references are directed to balanced lethal systems that are employed to maintain a plasmid in a population of cells. There is no disclosure of a control mechanism as required by the instant claims. Nor would such a control mechanism serve to advance the purpose of a balanced lethal system. The balanced lethal system requires only that the essential gene be expressed. The essential gene in the cited references is expressed regardless of the environment the host cell is in. This is a critical distinction, and renders the instant claims patentably distinct from the cited references. In light of the above discussion, applicants respectfully request reconsideration and withdrawal of these rejections.

Claims 30-33, 35-38, 48-49 and 65 are rejected under 35 U.S.C. §102(e) as being anticipated by Curtiss III (U.S. Patent No. 5,672,345). That patent is cited as teaching a method utilizing an *Asd*-negative mutant that can express a heterologous antigen. The cited reference is directed to a balanced lethal system for maintaining a recombinant gene in a population of cells. There is no disclosure of a mechanism by which expression of the *asd* gene is expressed when the cell is in an animal and not expressed when the bacterial cell is outside of the animal. There is no suggestion of such a mechanism because such a mechanism would not contribute to the effectiveness of the balanced lethal system for maintaining the plasmid in the bacterial cell. The critical limitation of the instant claims, namely the controlled expression of the essential gene, is not taught or even suggested by the cited reference. In light of the above discussion, applicants respectfully request reconsideration and withdrawal of these rejections.

Claims 30-33, 35-38 and 65 are rejected under 35 U.S.C. §102(b) as being anticipated by Jagusztyn-Krynicka et al. (1993). That reference is cited as teaching a method of administering a bacterial cell that comprises expression antigens and wherein the expression vector also comprises an *asd*+ gene to complement a mutated chromosomal *asd* gene. This system is also a balanced lethal system as discussed above. Jagusztyn-Krynicka et al provides no disclosure or suggestion of differential expression of the *asd*+ gene based on environmental factors. Again, a critical feature of the instant claims is that the bacteria comprises an essential gene under a mechanism of control such that the essential gene is expressed when the bacteria is inside of the animal and not expressed when the bacteria is outside of the animal. This insures that if the bacteria is, for example, excreted, it will die. Thus, the environmentally limited viability system operates to kill the bacteria when it is not in the desired permissive environment. The cited reference simply does not teach or suggest such a limitation. In light of the above discussion, applicants respectfully request reconsideration and withdrawal of these rejections.

Claims 30-33, 35-38 and 65 are rejected under 35 U.S.C. §102(b) as being anticipated by Gentry-Weeks et al. (1992). That reference is cited as teaching a method of administering an avirulent bacterial cell that comprises an expression antigen wherein loss of the *Asd*+ plasmid would result in cell death outside the animal in the absence of DAP. Again, the instant claims contain a critical limitation that is not disclosed by the cited reference. The reference is directed to a balanced lethal system for maintenance of a recombinant plasmid within a population of cells. The statement in the Office Action that the "loss of the *Asd*+ plasmid would be in the absence of DAP resulting in cell death outside the host animal" may be true, but is not relevant. Gentry-Weeks et al. does not disclose the claim limitation where the essential gene is not expressed when outside of the animal. The concept of the system disclosed in that reference is that the bacterial cell will die in the absence of the *Asd*+ plasmid. In this way, the surviving population is insured of containing the plasmid. The methods disclosed therein address a completely different issue than does the instant invention. There is no disclosure of, or suggestion of, an environmentally limited viability system as claimed. The critical feature of the instant invention is that the essential gene is expressed when the bacteria is inside of the animal and is not expressed when the bacteria is outside of the animal, such that the bacteria is viable when it is inside of the animal, but dies when it is outside of the animal. This limitation is simply not taught or suggested by the cited reference. In light of the above discussion, applicants respectfully request reconsideration and withdrawal of these rejections.

Claims 30-33, 35-38 and 65 are rejected under 35 U.S.C. §102(b) as being anticipated by Schodel et al. (1994). That reference is cited as teaching a method of administering an avirulent microbial cell that comprises an antigen, where loss of the *Asd*+ plasmid would result in death of the

microbe. As in the references cited above, this reference also fails to teach or suggest at least one critical limitation of the instant claims. There is no teaching or suggestion in Schodel et al. to control expression of the essential gene, *asd*, such that it is expressed when the microbe is inside of the animal and is not expressed when the microbe is outside of the animal. The utility of the system disclosed in that reference is completely different than the instant invention. The *asd* gene in that system is part of a balanced lethal mechanism designed to insure that the expression plasmid is retained in a population of cells. Therefore, there is no reason to control the expression of the *asd* gene. In light of the above discussion, applicants respectfully request reconsideration and withdrawal of these rejections.

Claims 30-33, 35-38 and 65 are rejected under 35 U.S.C. §102(b) as being anticipated by Cieslak et al. (1993). Cieslak is cited as teaching a method of administering an avirulent microbial cell comprising an expression antigen, where the loss of a plasmid containing *Asd+* results in death of the cell. As above, this reference also fails to teach or suggest the limitation of the instant claims that the essential gene is expressed when the bacteria is inside of the animal but not when the bacteria is outside of the animal. Cieslak et al. address the problem of retaining an expression plasmid in a population of cells, and therefore their system would not benefit from the controlled expression of the *asd* gene required by the instant claims. The present invention is directed to a system of biological containment, wherein differential expression of the essential gene is critical. With respect to Cieslak, as well as all of the above-cited references, the Office Action states that "the loss of the *asd+* plasmid would be in the absence of DAP resulting in cell death outside the host animal." While this statement is correct, it is also incomplete. The loss of the *asd+* plasmid would result in death of the bacterial host cell regardless of whether the cell is inside or outside of the animal. Such a system has absolutely no utility as a means of biological containment. The *asd+* gene is still expressed whether the bacterial cell is inside or outside of the animal. There simply is no control mechanism associated with the *asd* gene. The methods of the instant claims are clearly different than all of the cited references in that controlled expression of the essential (i.e., *asd*) gene is absolutely critical to to the ELVS of the instant claims for achieving biological containment of the bacterial host cell. In light of the above discussion, applicants respectfully request reconsideration and withdrawal of these rejections.

Rejections under 35 U.S.C. §103(a)

Claims 30-33, 35-38, 50, 52-54 and 65 are rejected under 35 U.S.C. §103(a) as being obvious over either one of Curtiss III (U.S. Patent No. 5,672,345) or Curtiss III et al. (1989) in view of Molin et al. (U.S. Patent No. 5,702,916). The Curtiss references are cited as above with respect to §102 rejections. Molin et al. is cited as teaching a means of constructing microbial cells that comprise both essential and lethal genes in an environmentally limited viability system, with the use of temperature

sensitive promoters or repressors, for the purpose of producing recombinant host cells that are growth inhibited when outside the animal host and useful for inducing an immune response in an animal.

The discussion of the Curtiss III references above applies here as well. Again, both fail to teach a critical limitation of the instant claims. With respect to Molin, applicants contend that as amended, the instant claims are not made obvious by that reference, because the essential genes of the present invention are copies of native genes, or are essential for metabolism, growth, cell wall integrity or cell membrane integrity of the bacterial cell, as defined at pages 12-16 of the specification. This approach was not taught or suggested by Molin. In the methods disclosed in that reference, the *hok* and *sok* genes are taught as lethal and essential genes, respectively. The principle of that system is that when the host cell is in a permissive environment, i.e. inside of the animal, the *hok* gene expresses a cell killing function that is suppressed by expression of the *sok* gene, which encodes an mRNA sequence that acts as an antisense sequence with respect to the *hok* mRNA. There is a differential half-life for the two mRNAs, such that when the plasmid containing both genes is lost from the host cell, the *hok* mRNA is eventually translated and thus the cell is killed, because the *sok* mRNA is no longer present in inhibitory concentration. Expression of either the *hok* or *sok* genes may be accomplished, for example, by linking with a promoter that is responsive to changes in certain environmental conditions. The system described by Molin et al. differs from the claimed system because the essential gene described by Molin is only essential in that it inhibits translation of mRNA from the lethal gene, *hok*. In this context, the system functions to inhibit expression of the cell killing function of *hok* when the host cell is in a permissive environment. Thus, in the absence of the lethal *hok* gene, the expression of *sok* would have no effect on the survival of the host cell, whether that host cell was inside of or outside of an animal. This is in contrast to the system of the instant claims, in which the essential gene operates independently from the lethal gene. In fact, there is no requirement that the system of the instant claims even employ a lethal gene to be effective.

Applicants further contend that the claims as amended are distinguishable from the teachings of Molin in that the essential gene of the instant claims is a copy of a native gene or is essential for metabolism, growth, cell wall integrity or cell membrane integrity of the bacterial cell. The *sok* gene of Molin is not a native gene, nor is it essential for metabolism, growth, cell wall integrity or cell membrane integrity. Nor is there any suggestion to use such a gene. The system of Molin is directed to regulatable expression of a cell-killing gene, such as *hok*. One of the mechanisms suggested by Molin for regulating the cell killing function of *hok* is to regulate that function at the level of translation. Since *sok* essentially encodes an antisense RNA to the mRNA of *hok*, it has potential utility in such a mechanism. The mechanism essentially comprises differentially expressing the mRNAs of *hok* and *sok* based on the environmental conditions that the host cell is exposed to. This can be accomplished either by up-

regulating expression of *hok* mRNA relative to *sok* mRNA, or by down-regulating *sok* mRNA relative to *hok* mRNA, in a non-permissive environment. Thus, the essential gene of Molin is only essential when the host cell also harbors the lethal gene, *hok*. Molin et al. does not teach or suggest regulating expression of an essential *native* gene as a means of biological containment. Nor does Molin et al. teach or suggest regulating expression of an essential gene that is *essential for metabolism, growth, cell wall integrity or cell membrane integrity of the bacterial cell* as a means of biological containment. As each element of the claims is not disclosed by the cited references, and as there is no suggestion or motivation to combine them, the requirements for prima facie obviousness have not been met. Thus, the claims of the instant application are not made obvious by the combination of references cited.

In light of the foregoing, applicants respectfully request reconsideration and withdrawal of these rejections.

Claims 30-33, 35-38, 50, 52 and 65 are rejected under 35 U.S.C. §103(a) as being unpatentable over either Curtiss III (U.S. Patent No. 5,672,345) or Curtiss III (1989) in view of Miller et al. (U.S. Patent No. 6,090,901). Miller is cited as teaching the importance of using environmentally regulated promoters for the controlled expression of an antigen, such that the antigen is only expressed in selected cells or cellular compartments.

The Curtiss references are discussed above, relative to §102 rejections, and those arguments apply here as well. With respect to the Miller reference, there is no suggestion to use such environmental regulation to provide an environmentally limited viability system as claimed. That reference is directed to expressing an antigen only in certain environmental conditions. The system discussed therein does not relate to biological containment of a microorganism. The microorganism taught in Miller would survive whether the organism is inside of an animal or outside of an animal. The host organism survives under any conditions in which the wild type organism would survive. As such, there is no biological containment of the microorganism. The system of Miller is useful only in preventing a heterologous antigen from being expressed in non-permissive environments. There is no suggestion in Miller or either Curtiss III reference to use the promoter of Miller to control expression of an essential gene for the purpose of limiting the viability of the host cell in non-permissive environments. All of the elements of the instant claims are not taught by the combination of references. The critical limitation of the instant claims, that the essential gene is expressed in a permissive environment but not in a non-permissive environment is simply not taught by any of the cited references. As all of the elements of the instant claims are not taught by the cited combination of references, and because there is no suggestion to combine the teachings of the cited references because they are directed to solving different problems, the requirements for prima facie obviousness have not been met.

In light of the foregoing, applicants respectfully request reconsideration and withdrawal of the rejections.

Claims 30-33, 35-38, 47-49 and 65 are rejected under 35 U.S.C. §103(a) as being unpatentable over either Curtiss III ('345 patent) or Curtiss III (1989) in view of Curtiss III (U.S. Patent No. 4,968,619). Curtiss '345 and 1989 are cited as above, while Curtiss III ('619) is cited as teaching the use of an environmentally controlled promoter to limit the expression of a heterologous antigen, such that it is expressed in permissive environments (i.e. inside of an animal) and is not expressed in non-permissive environments (i.e. at room temperature, such as during preparation or storage of the vaccine). That reference also teaches the use of an essential gene to stabilize the vector in a population of host cells. There is no teaching in any of the cited references of the use of an environmentally controlled promoter linked to the essential gene. In all of those references, the essential gene is expressed regardless of the environment that the host cell is in. There is no suggestion or motivation to use such regulation, because the function of those systems would not be improved by regulation of the essential gene. As all of the elements of the instant claims are not taught by the cited references, nor is there a suggestion or motivation to combine their teachings, the requirements for a prima facie finding of obviousness have not been met.

In light of the foregoing, applicants respectfully request reconsideration and withdrawal of these rejections.

Claims 30-33, 35-38, 50, 52, 53 and 65 are rejected under 35 U.S.C. §103(a) as being unpatentable over Curtiss III ('345) in view of Molin et al. ('916) and Hershberger et al. Curtiss III and Molin are cited as above. Hershberger is cited as showing an extra chromosomal vector that comprises a cI857 repressor for the purpose of stabilizing and selecting recombinant DNA host cells through the use of a lethal marker and complementary cloning vectors. As with the Curtiss III reference discussed above, Hershberger is directed to a method of stabilizing a recombinant vector in a population of host cells. The specification of that patent teaches that a cell constructed with a lethal marker on a chromosome, and a repressor on a recombinant vector, will die if it loses the vector. This is essentially a balanced-lethal system, which has no utility whatsoever for biological containment of the host cell. The host cell taught by that reference would survive in any environment that the wild-type cell would survive in, as long as the cell retained the recombinant vector comprising a repressor. Applicants point out that the claims as amended herein require that the bacterial cell comprise an environmentally limited viability system comprised of an essential gene that is a copy of a native gene, or a gene that is essential for metabolism, growth, cell wall integrity or cell membrane integrity of the bacterial cell, wherein the essential gene is expressed when the cell is in the permissive environment (in the animal) and not expressed when the cell is in a non-permissive environment (outside of the animal). None of the cited references teach this

limitation. The systems of Hershberger and Curtiss III would not benefit from controlled expression of the gene, because the systems function optimally when the gene is expressed under all environmental conditions. Molin is discussed above, and the arguments provided there are applicable here as well. Molin does not teach regulated expression of an essential gene wherein the essential gene is a copy of a native gene. There would be no motivation to combine the teachings, because a balanced lethal system for retention of a vector in a population of host cells would not benefit from controlled expression of the essential gene. Likewise, the system of Molin would not benefit from the use of an essential gene that is a copy of a native gene because the use of an essential gene in that context is as a means of regulating the cell killing function of a lethal gene. None of the references disclose an essential gene, other than *sok* (which is a non-native gene, and is not essential for metabolism, growth, cell wall integrity or cell membrane integrity of the bacterial cell), that is under the control of an environmentally regulated expression mechanism. As all of the elements of the claims are not taught by the cited references, and because there is no suggestion or motivation to combine the references, the requirements for establishing prima facie obviousness have not been met.

In light of the foregoing, applicants respectfully request reconsideration and withdrawal of these rejections.

Claims 30, 33, 36-44, 46 and 52 are rejected under 35 U.S.C. §103(a) as being unpatentable over Curtiss III (U.S. Patent No. 5,840,483) in view of Youderian (1980). Curtiss III ('483 is cited as teaching a bacterial cell that is delta *asd* and comprises P22 bacteriophage genes, along with an extrachromosomal copy of the *asd* gene. Youderian is cited as teaching that genes 13 and 19 of P22 encode lysogenic products.

Curtiss III ('483) discloses a balanced lethal system, as do the other Curtiss III references, directed at stabilizing a host vector in a host cell population. In such a system, there is no environmental control mechanism associated with the *asd* gene. The function of the system described therein does not require, nor would it benefit from, such a control mechanism. The specific strain cited, χ3115, does not comprise a control mechanism whereby *asd* is expressed in a permissive environment and is not expressed in a non-permissive environment, as is required by the instant claims. The statement at page 19 of the Office Action, that Curtiss III ('483) teaches "an extrachromosomal gene that encodes the essential gene and stabilizes the microbial cell while in the host but would not be stable outside the animal" is simply false. The microbial cell disclosed comprises an extrachromosomal gene that encodes *asd*. Thus, the extrachromosomal vector carrying the *asd* gene is stabilized in the population of host cells because without that vector, the product of the *asd* gene, which is essential for cell viability, is absent. This is true whether the host cell is inside of the animal or outside of the animal, because there is no control mechanism associated with the extrachromosomal *asd* gene. Thus, all of the elements of the claims are

not disclosed in the cited references. In addition, there is no suggestion to combine the cited combination because the system of Curtiss III (a balanced-lethal system) does not benefit from controlled expression of the *asd* gene. Thus, the requirements for establishing prima facie obviousness have not been met. Applicants therefore respectfully request reconsideration and withdrawal of these rejections.

Conclusions:

The claims as amended are in condition for allowance. In light of the above discussion, applicants respectfully request such action. If any issues remain, applicants request that the undersigned be contacted.

Respectfully submitted,

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March 6, 2001